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HEPATOPANCREAS OF *PARATELPHUSA HYDRODROMOUS* (HERBST): HISTOPHYSIOLOGY AND THE PATTERN OF PROTEINS IN RELATION TO REPRODUCTION AND MOLT

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A major seat of bulk storage, syntheses and transformations of a variety of organic and inorganic substances, the hepatopancreas of crustaceans, which is analogous to the liver of vertebrates and the fat body of insects, may aptly be described as a metabolic factory *par excellence* (Lockwood, 1967; Huggins and Munday, 1968; Adiyodi and Adiyodi, 1970). Hepatopancreatic reserves, which are maximal in intermolt (stage C₄) are mobilized to meet the demands of somatic and reproductive growths, and also exigencies like inanition and perhaps also altered environmental conditions. The free sugars (Adiyodi and Adiyodi, 1970a) and phospholipids (Adiyodi and Adiyodi, 1970b) in the hepatopancreas of the crab, *Paratelphusa* show variations, both qualitative and quantitative, related to vitellogenesis, suggesting that these substances may be utilized in some way in the formation of yolk. Cyclic fluctuations occur in the levels of organic (Adiyodi, 1969a) and inorganic (Adiyodi, 1969b) reserves in the hepatopancreas of *Paratelphusa* associated with the periodic molt. Our earlier studies have shown that the major organic resources in the hepatopancreas of *Paratelphusa* are lipid (Adiyodi, 1969a) and free sugars (Adiyodi and Adiyodi, 1970a). Of the constituents analyzed, the neutral fats (Adiyodi, 1969a) and phospholipids (Adiyodi and Adiyodi, 1970b) predominate among the lipid, the free unsaturated fatty acids and cholesterol occurring in medium and low levels, respectively (Adiyodi and Adiyodi, 1972).

In insects a dynamic relationship exists between the hemolymph and the fat body proteins (Chippendale and Kilby, 1969). Changes have been described in the profiles and proportions of proteins in the plasma of *Paratelphusa* (see Adiyodi and Adiyodi, 1970, for references) and several other species of decapod Crustacea, related to molt and reproduction (Barlow and Ridgeway, 1969; Kerr, 1969). The hepatopancreas has been implicated in the synthesis of proteins in Decapoda (Kurup and Scheer, 1966). But the nature of the relationship existing between proteins in the hepatopancreas and the blood has been little investigated. Our

knowledge on the histology, ultrastructure and storage function of the hepatopancreas is also meagre in the Decapoda, except perhaps in the crayfish, *Procambarus clarkii* (Ogura, 1959; Miyawaki, Matsuzaki and Sasaki, 1961; Miyawaki and Tanoue, 1962). This study has as its main objectives the exploration of the nature and extent of protein synthesis and "storage" in the hepatopancreas, inter-relationship between these proteins and those of the plasma, their utilization in molt and reproduction, their fate during starvation, and the influence of eyestalk hormone or hormones on their accumulation and utilization. The overall role of the hepatopancreas of *Paratclphusa* as a synthesis-depository-supply center of the major organic resources is also discussed with special reference to the demands of somatic and reproductive growths.

MATERIALS AND METHODS

Material

The crabs used were mostly fresh from paddy fields, and included juveniles and adults of both sexes. The stage in the molt cycle was determined according to Drach's (1939) schedule. Yolk deposition (which has been used as a parameter in our studies) may be divided into two phases, Vitellogenesis I (subdivided into stages 1-3) and Vitellogenesis II. Stage 1 oocytes are white in color; stage 2 oocytes are a pale yellow, and show the beginnings of protein yolk synthesis. Stage 3 oocytes acquire an orange color and reach an average diameter of 1 mm. Vitellogenesis II, which follows stage 3 is a period of rapid yolk deposition, terminating eventually in oviposition.

Histochemical and biochemical procedures

The hepatopancreatic lobules of crabs in different stages of the molt cycle were fixed in 10% neutral formalin or chilled absolute alcohol. The dehydration of tissues fixed in the latter was carried out at temperatures ranging from 3-5° C. Paraffin sections, 5 μ in thickness, were stained with Millon's reagent (Baker modification), mercury-bromophenol blue (after Bonhag) (MBB), Sudan black B (after McManus) (SBB), and periodic acid-Schiff (Lillie's method) (PAS) (Pearse, 1968). Millon's, xanthoproteic and biuret tests were employed for biochemical detection of proteins in water homogenates of hepatopancreatic tissues (Oser, 1965).

Electrophoresis

Soluble proteins of the hepatopancreas, ovary and blood of crabs in different stages of molt cycle and reproductive activity were analyzed qualitatively by disc electrophoresis on polyacrylamide gels. The ovary and hepatopancreas were removed from crabs, which had been prechilled for 10 minutes, into cold Ringer. They were washed well with glass distilled water and homogenized in an all glass homogenizer. The homogenates were centrifuged in the cold (3-5° C) at 5000 rev/minute for 20 minutes. Eight μ l of the supernatant was used for the fractionations.

Electrophoresis was conducted in a Canalco Model 6 Disc Electrophoretic Unit for 20 minutes under a constant current of 6 mamp/column using β alanine acetate

as the buffer (pH 4.5). In the case of the hemolymph, the cellular elements were spun off by centrifugation in the cold (3–5° C), and only the clear plasma (8 μ l) used. After electrophoresis the gels were immersed in a 0.1% solution of amido black (AB) in 7.5% acetic acid (2 hours). Background stain was removed electrophoretically (10 mamp/gel). For the detection of lipoproteins the gels were immersed in a saturated filtered solution of SBB in 70% ethanol for 24 hours, destained in 70% ethanol, and transferred to 7.5% acetic acid for hydration and storage. For glycoproteins, the gels were first placed for 1 hour in 7.5% acetic acid soon after electrophoresis, and for another hour in 0.2% periodic acid (4° C); after a short rinse in 7½% acetic acid they were transferred to Schiff's reagent (4° C) for 12 hours (PAS). Fractions have been numbered serially from the origin, based on their relative mobilities (R_{mb}) in the electric field. In the nomenclature employed here, II, P and OV denote proteins of the hepatopancreas, plasma and ovary, respectively.

Eyestalk removal

Eyestalks were removed from juvenile crabs in stage C₄ and a few adults, one eyestalk at a time, with an interval of 24 hours between the two operations on each individual, with a view to study the influence of eyestalk hormones on protein synthesis and utilization. This method of operation reduced the postoperative mortality to about 5%. The experimentals and controls (normal crabs) were maintained on a diet of earthworms (*Megascolex* sp.).

Starvation

Seven adult female crabs in their intermolt were individually reared in glass jars for 3 months on a bed of moist filtered sand. The crabs had ready access to water, but no food was provided. At the beginning of the experiment, the stage of the ovary was ascertained by the window method (Gomez and Nayar, 1965).

RESULTS

Histology and histochemistry of the hepatopancreas

In normal well-fed adult female crabs nearly three-fourths of the body cavity is filled by the hepatopancreas. Usually it has an orange hue, but its color may change with the stages in the molt cycle. Hepatopancreas is deep brown or black in postmolt, orange in intermolt (stage C₄) and black in late premolt. Such color differences could hardly be detected in starved or ill fed crabs, the hepatopancreas of which appeared a dull pale yellow. The hepatopancreas of some of the destalked crabs became whitish on molt, despite the fact that the animals were well cared for during the postoperative period. Possibly, this is related to the fact that the crabs become hypophagic after the initial hyperphagia on eyestalk removal, and the hepatopancreatic reserves become consequently much depleted.

Longitudinal sections of the hepatopancreatic lobules of *Paratclphusa* show the presence of three separate zones, as already briefly reported (Adiyodi, 1969a). Zone I is composed of diminutive, closely packed embryonic cells, and Zone II largely of secretory cells or Blastenzellen. In Zone III, which constitutes the major portion of the lobule, two types of cells corresponding to the metal cells

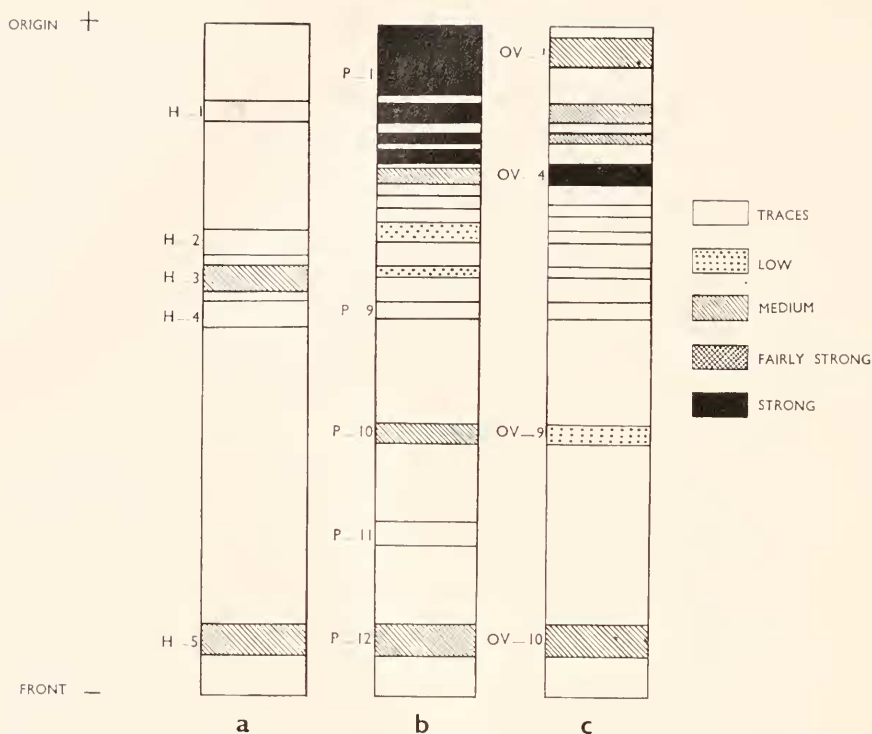


FIGURE 1. Electropherograms of *Paratelphusa hydrodromous* showing the pattern and homology of proteins resolved at pH 4.5 in different tissues; (a) hepatopancreas (diagrammatic showing all the fractions); (b) plasma (in stage 3 of Vitellogenesis I) and (c) ovary (in stage 2 of Vitellogenesis I).

(iron cells and copper cells) described in the midgut gland of the crayfish, *Procambarus clarkii* by Ogura (1959) and also Miyawaki, Matsuzaki and Sasaki (1961) are observed. The iron cells of *Paratelphusa* have a nucleus roughly 7μ in diameter and a large nucleolus; the cytoplasm is rather homogeneous and strongly basophilic. Copper cells have smaller nuclei (about 4μ in diameter) and vacuolated cytoplasm, the vacuoles often being filled with stainable substances. The metal cells in Zone III serve as storage centers of both organic and inorganic (*e.g.*, calcium) reserves.

During stage C_4 the interior of the metal cells is literally filled with substances positive to SBB and PAS. With MBB and Millon's, on the other hand, the general stainability of the metal cells was very low even in stage C_4 . Distinct droplets strongly positive to MBB and Millon's were observed in small amounts in the interlobular spaces, but very rarely inside the metal cells. These droplets were positive also to SBB and PAS. The observations reported here indicate a low level of stainable protein in the hepatopancreatic tissue. This was further substantiated by the biochemical tests for proteins (Millon's, xanthoproteic and biuret reactions) on whole tissue homogenates.

Soluble proteins of the hepatopancreas in relation to reproduction

Electropherograms of the hepatopancreas showed a total of five protein fractions (H-1 to H-5) (Fig. 1a). The plasma samples of female crabs with ovaries in stage 3 run simultaneously under the same system for reference and comparison showed 12 fractions (P-1 to P-12) (Fig. 1b). Stage 2 ovaries showed the maximum number of protein fractions (10) (OV-1 to OV-10) (Fig. 1c). Fractions OV-1 to OV-3 appear to be homologous in their R_{mbs} to P-1 to P-3, respectively, OV-4 to both P-4 and P-5, OV-5 to OV-9, respectively, to P-6 to P-10, and OV-10 to P-12. Fractions H-1 to H-5 of the hepatopancreas were found to correspond in their R_{mbs} to fractions P-2, P-7, P-8, P-9 and P-12 of the plasma, respectively, and to fractions OV-2, OV-6, OV-7, OV-8 and OV-10 of the ovary, respectively. The fastest moving fraction H-5 resolved in some gels as a heterogeneous chromoprotein. Reaction with SBB and PAS was negative at all stages of the reproductive cycle suggesting that fractions H-1 to H-4 are only simple proteins.

Homogenates of the hepatopancreas of juvenile females (Fig. 2a) and adult males (Fig. 2b) yielded during the internolt generally two AB-stainable fractions, H-3 and H-5, the former only in extremely low levels. The hepatopancreas of juvenile males (Fig. 3c) in their internolt yielded sometimes besides these fractions also fraction H-2, though in traces.

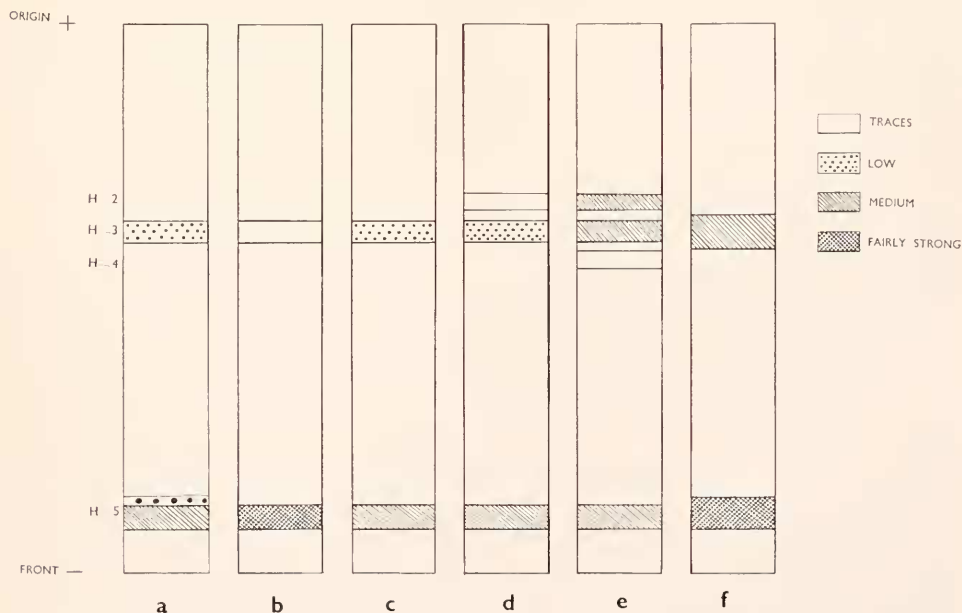


FIGURE 2. Electropherograms of the hepatopancreas of *Paratellphusa hydrodromous*; (a) juvenile females in stage C_4 ; (b) adult males in stage C_4 ; (c) adult females with the ovaries in stage 1; (d) adult females with the ovaries in stage 2; (e) adult females with the ovaries in early stage 3 and (f) adult females with the ovaries in mid and late stage 3 of Vitellogenesis I.

the hepatopancreas of intermolt adult females with their ovaries in stage 1 (Fig. 2c) was comparable to that of juvenile females and adult males. Fraction H-2 appeared with stage 2 (Fig. 2d). With the ovaries in early stage 3 (Fig. 2e) four fractions could be detected in the hepatopancreas (H-2 to H-5); but in mid-stage 3 individual fractions, H-2 and H-4, were hardly distinguishable (Fig. 2f). Mid and late stage 3 crabs had more AB-stainable proteins in fractions H-3 and H-5 than forms in early stage 3.

Soluble proteins of the hepatopancreas in relation to the molt schedule

Only juvenile crabs were used for these studies. The hepatopancreatic electropherograms of early (stage A and B) (Fig. 3a) and late postmolt (stages C_1 – C_3) (Fig. 3b) crabs were remarkably similar to those obtained in intermolt (stage C_4) (Fig. 2a). In juvenile females the biggest peak was represented during stage C_4 by the fast moving fraction (H-5); fraction H-3, the other component present, was in traces. There occurs a decrease in the concentration of fraction H-3 during early premolt (D_0 – D_1) (Fig. 3d). As the crabs entered D_2 – D_3 this fraction registered a sharp increase (Fig. 3e). Late premolt (stage D_4) was characterized by the appearance of a slow-moving high molecular protein (fraction H-1), not hitherto represented in the electropherograms of the hepatopancreas (Fig. 3f). In some crabs this was attended with a slight fall in the levels of fraction H-5.

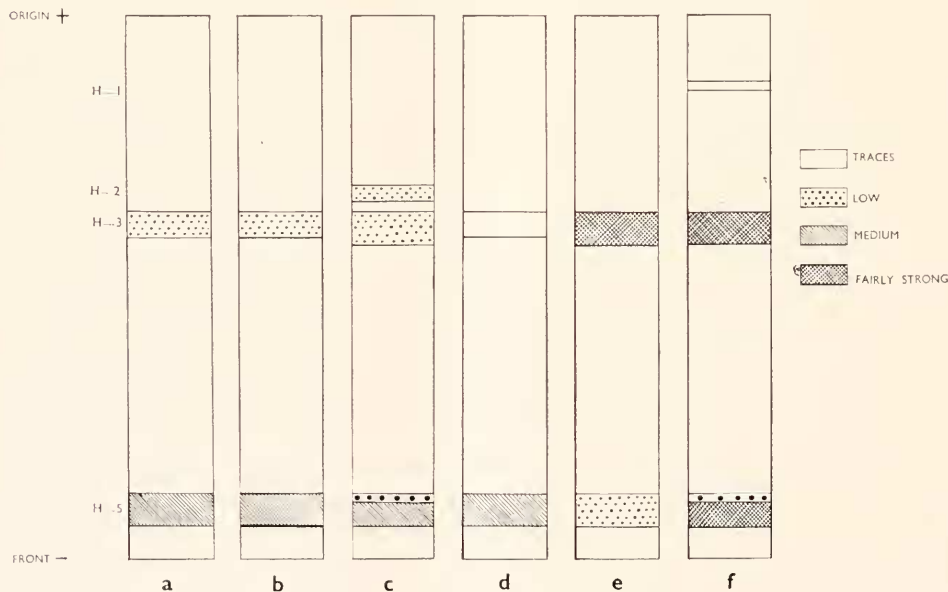


FIGURE 3. Electropherograms of the hepatopancreas of *Paratclphusa hydrodromous* in relation to the molt schedule; (a) juvenile females in stage A–B; (b) juvenile females in stage C_1 – C_3 ; (c) juvenile male in stage C_4 ; (d) juvenile females in stage D_0 – D_1 ; (e) juvenile females in stage D_2 – D_3 and (f) juvenile females in stage D_4 .

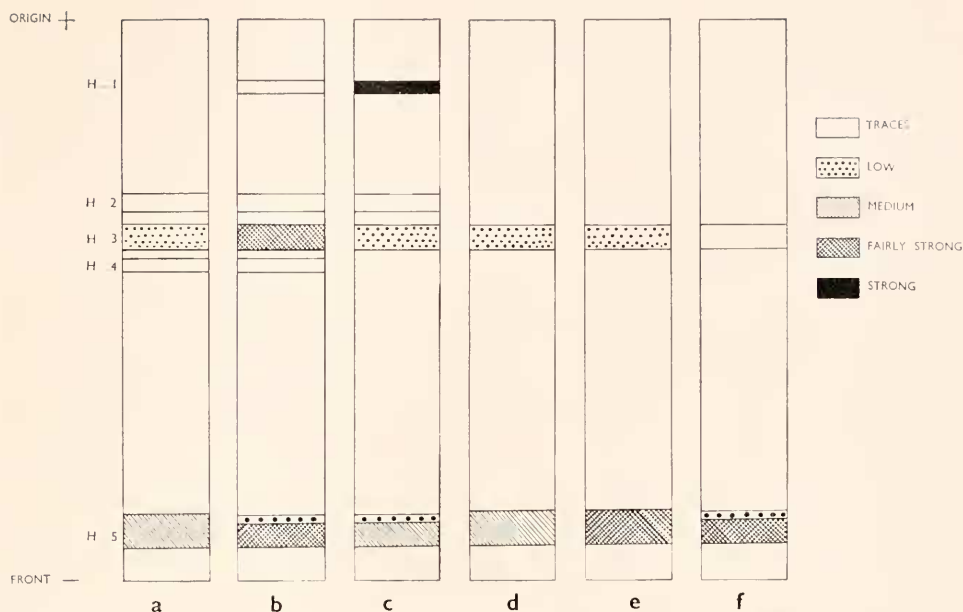


FIGURE 4. Electropherograms of the hepatopancreas of *Paratelphusa hydrodromous* under eyestalk ablation; (a) juvenile female D_0 - D_1 , 8 days after eyestalk removal; (b) juvenile female in D_3 - D_4 , 15 days after eyestalk ablation; (c) adult female, 8 days after eyestalk removal; (d) juvenile female (control) after 15 days; (e) adult male, 15 days after eyestalk ablation and (f) destalked adult female administered eyestalk extract, 3 days after the operation and assayed 5 days thereafter.

Pattern of proteins in the hepatopancreas of destalked crabs

Homogenates of the hepatopancreas of 7 juvenile female crabs were analyzed electrophoretically, 3 on the 8th day and the remaining 4 on the 15th day of ablation of both the eyestalks. Some of the electropherograms showed all the 5 fractions (H-1 to H-5), fraction H-1 being present particularly in forms that had precipitously entered D_3 - D_4 (Fig. 4b). The crabs in D_0 - D_1 showed only fractions H-2 to H-5 (Fig. 4a). Four adult female crabs with ovaries in stage 1 were deprived of their eyestalks, and their hepatopancreas assayed for proteins 8 days after the operation (Fig. 4c). They were in stage C_4 of the molt cycle at autopsy, but showed a larger number of fractions than the 3 control adult female crabs, and also an accelerated entry into late stage 2 or early stage 3 of vitellogenesis. The pattern in destalked adult females simulated in some respects that of crabs with ovaries in early stage 3, but more so was the comparison with the picture obtaining in premolt juvenile crabs in that fraction H-1 was also present. None of the 5 control juvenile females (with intact eyestalks) entered premolt, the only fractions in their hepatopancreas being H-3 and H-5 (Fig. 4d). The electropherograms of the hepatopancreas of two adult males (Fig. 4e), 15 days after eyestalk removal showed, like those of their 2 controls, only two fractions, H-3 and H-5, these destalked male crabs were still in stage C_4 .

It was necessary to ascertain whether the effects observed by eyestalk displacement on hepatopancreatic proteins could be reversed by substitution therapy. For this purpose an extract of eyestalks of intermolt adult females was prepared in Ringer and injected into 3 females having stage 1 ovaries, at the rate of two eyestalks in 0.01 ml Ringer/crab, 3 days after their eyestalks had been removed. The hepatopancreas was assayed 5 days after the administration of the extract. The hepatopancreatic electropherograms of the destalked females which received the eyestalk extract (Fig. 4f) were almost indistinguishably like those of normal intermolt adult females with stage 1 ovaries (Fig. 2c) in that there were only two fractions, H-3 and H-5.

Effect of starvation on hepatopancreatic soluble proteins

All the crabs subjected to inanition had their ovaries at the beginning of the experiment at stage 3 (early, mid and late) of vitellogenesis I. Prolonged food withdrawal led not only to an arrest in oocyte growth, but also to yolk lysis and oosorption in as many as 5 out of the 7 crabs. The hepatopancreas of the starved individuals wore a bleak paper-white appearance and showed much reduction in size and weight. Electropherograms of the hepatopancreas of animals with resorbing ovaries showed mostly only fraction H-5 (fraction H-3 being often too low to be detected), whereas in the two crabs with no visible signs of resorption, fraction H-3 was present in medium levels.

DISCUSSION

It is well known that large amounts of organic resources accumulate in the hepatopancreas of crustaceans during the intermolt, and that such stores are mobilized to meet the demands of events like molt and reproduction (Adiyodi and Adiyodi, 1970). Though estimates of total protein content are not available, our histochemical and biochemical studies sufficiently indicate that the protein level in the hepatopancreas of *Paratelphusa* is rather low even in the intermolt. The hepatopancreas of *Paratelphusa* is rich in monosaccharides (glucose, galactose) and the disaccharide, sucrose (Adiyodi and Adiyodi, 1970a). The intensity of color developed with PAS, it may be recalled, is dependent primarily on the amount of reactive glycol structure present, the concerned reactive groups being those of the hexose sugars, glucose, galactose, mannose and the methylpentose sugar, fucose (Pearse, 1968). The hepatopancreas of *Paratelphusa* is also rich in phospholipids especially phosphatidyl choline, phosphatidyl ethanolamine and lysophosphatidyl ethanolamine (Adiyodi and Adiyodi, 1970b). The hepatopancreatic lipid of this crab is semiliquid on extraction suggesting the presence of fairly high quantities of unsaturated fatty acids; notable among the nonesterified fatty acids being oleic/palmitoleic acid (Adiyodi and Adiyodi, 1972). The unsaturated fatty acids and the first two phospholipids also react positively with PAS (Pearse, 1968). The abundantly positive color reaction shown by the metal cells of *Paratelphusa* to PAS may possibly be, therefore, attributed to sugars, phospholipids and unsaturated fatty acids rather than glycogen, which was very low to be detected with Best's carmine reaction or iodine test (Adiyodi and Adiyodi, 1970a). It is not known whether the PAS-positive substances observed in our histochemical preparations included also some glycoprotein not resolved with the acidic buffer system we have used.

A comparison of the patterns of soluble proteins in the hepatopancreas, plasma and ovary (Fig. 1) and their changes in relation to the reproductive (Fig. 2) and molt (Fig. 3) cycles (Adiyodi and Adiyodi, 1970, for references) permits some conclusions. Fractions corresponding to the vitellogenins (P-1 to P-5 or OV-1 to OV-4) were not represented in the hepatopancreas, except for the relatively minor fraction H-1, which is homologous to P-2 of the plasma and OV-2 of the ovary. Further, fraction H-1 is an unconjugated protein, whereas in the plasma its homologue, P-2 is in the form of a glycoprotein in stage 2, and in late periods of vitellogenesis; in the ovary OV-2 assumes the characteristics of a glycoprotein in late stage 3 and in Vitellogenesis II (Adiyodi, 1968a). The possibility that fraction H-1 may contribute to the plasma fraction P-2 in *Paratelphusa* can hardly be discounted. But our studies clearly suggest that the plasma sex protein (P-4/5) which contributes significantly to yolk formation as also the other vitellogenins resolvable under acidic conditions of electrophoresis, are neither synthesized nor stored as such in the hepatopancreas. In this respect, the hepatopancreas of *Paratelphusa* differs from the vertebrate liver, which is the principal organ synthesizing serum albumin (Milhaud, 1964) and the insect fat body (Chippendale and Kilby, 1969), which synthesizes and supplies proteins to the ovary.

The increase in the levels of fraction H-3 with vitellogenesis, and the fact that fractions H-2 and H-4 make their appearance in adult females only in relation to specific stages in the ovarian cycle, suggest that these proteins may play some role in vitellogenesis, either as enzymes catalyzing some of the biological conversions, as carrier proteins in the plasma, or as fuel. The sudden decrease in the concentration of hepatopancreatic protein fractions H-3 and H-5 with the onset of premolt may perhaps be due to their increased utilization during this period of the formation of the new epicuticle. The appearance of fraction H-1 and the increase in the levels of soluble proteins in stage D₄ may be associated, at least in part, with the withdrawal of protein from the resorbing old cuticle into the hepatopancreas by way of the hemolymph. The appearance of additional plasma protein fractions or increased plasma protein levels associated with molt is already documented in insects (Siakotos, 1960) and crustaceans (Barlow and Ridgeway, 1969). In the land crab, *Gecarcinus lateralis*, according to Skinner (1965) the incorporation of valine-1-C¹⁴ and leucine-1-C¹⁴ into the hepatopancreatic proteins reaches its maximum by D₀-D₂. It is not known whether the proteins accumulated in the hepatopancreas of *Paratelphusa* in premolt include any enzymes related to molt or digestion. But the fact that in the molt cycle the protein fraction H-1 normally appears only associated with premolt points to a possible influence of the molt hormone on its accumulation.

The precocious appearance of additional fractions in intermolt juvenile and adult females consequent on eyestalk removal (which in adult females has been demonstrated to be readily reversible by substitution therapy) suggests that the synthesis of proteins in the hepatopancreas related to molt and reproduction are normally inhibited during the intermolt by some principles in the eyestalk, possibly the molt inhibiting and gonad inhibiting hormones. Such a view is further supported by the observation of McWhinnie and Mohrherr (1970) in the crayfish, *Orconectes virilis* that the eyestalk extract of intermolt animals reduces amino acid incorporation into the hepatopancreas of premolt crayfish. The fall observed in the levels of hepatopancreatic lipids (R. G. Adiyodi, unpublished) and sugars (Adiyodi and

Adiyodi, 1970a) in destalked *Paratelphusa* suggest that these substances are more readily pressed into service as fuels to meet the metabolic demands arising from eyestalk removal.

Starvation is known to reduce the rate of metabolism in Crustacea (Vernberg, 1959). In *Hemigrapsus* continued starvation for 23 days exercised little effect on glycogen content or lipid reserves, but in females there was a depletion of proteins (Neiland and Scheer, 1954). In *Paratelphusa* inanition for 3 months results in a fall in the lipid content of the hepatopancreas and a rise in PAS-positive substances, the latter possibly due to lipid breakdown (Adiyodi, 1968b). Though the observations reported here give no clue to the sequence in which the hepatopancreatic metabolites are utilized, they nevertheless indicate that the synthesis and/or accumulation of both lipid and protein become impaired. Possibly, the depletion observed in the hepatopancreatic protein with starvation may be related to its breakdown and drainage into other tissues including the hemolymph to counteract the stress on osmotic equilibrium.

We thank Prof. K. J. Joseph for his interest in the work.

SUMMARY

1. Protein level in the hepatopancreas of *Paratelphusa* is low even in intermolt. A total of 5 protein fractions (H-1 to H-5) could be detected under acidic conditions of disc electrophoresis. They are homologous to P-2, P-7, P-8, P-9 and P-12 of the plasma, and OV-2, OV-6, OV-7, OV-8 and OV-10 of the ovary, respectively, in their mobilities. None of the major vitellogenins including the plasma sex protein, which contributes significantly to yolk formation, is either synthesized or stored as such in the hepatopancreas. The increase in the levels of fraction H-3 with vitellogenesis, and the fact that fractions H-2 and H-4 appear in adult females only in relation to specific stages in the ovarian cycle, however, suggest that these proteins may play some role in vitellogenesis, as enzymes, carrier proteins or fuels.

2. Eyestalk removal causes in juvenile and adult females of *Paratelphusa* the addition of fractions H-1, H-2 and also H-4 to fractions H-3 and H-5 already present. The synthesis of the proteins in the hepatopancreas related to molt and reproduction appears to be normally inhibited during the intermolt by some eyestalk principle.

LITERATURE CITED

- ADIYODI, K. G., AND R. G. ADIYODI, 1970. Endocrine control of reproduction in decapod Crustacea. *Biol. Rev.*, **45**: 121-165.
- ADIYODI, R. G., 1968a. Protein metabolism in relation to reproduction and moulting in the crab, *Paratelphusa hydrodromous* (Herbst). Part II—Fate of conjugated proteins during vitellogenesis. *Indian J. Exp. Biol.*, **6**: 200-203.
- ADIYODI, R. G., 1968b. On reproduction and molting in the crab, *Paratelphusa hydrodromous*. *Physiol. Zool.*, **41**: 204-209.
- ADIYODI, R. G., 1969a. On storage and mobilization of organic resources in the hepatopancreas of a crab (*Paratelphusa hydrodromous*). *Experientia*, **25**: 43-44.

- ADIYODI, R. G., 1969b. Calcium cycle and the hepatopancreas in the freshwater crab, *Paratelphusa hydrodromous* (Herbst). *Journal of the Kerala Academy of Biology*, 1: 20-28.
- ADIYODI, R. G., AND K. G. ADIYODI, 1970a. Free sugars in *Paratelphusa hydrodromous* in relation to reproduction and molting. *Physiol. Zool.*, 43: 71-77.
- ADIYODI, R. G., AND K. G. ADIYODI, 1970b. Lipid metabolism in relation to reproduction and moulting in the crab, *Paratelphusa hydrodromous* (Herbst): Phospholipids and reproduction. *Indian J. Exp. Biol.*, 8: 222-223.
- ADIYODI, R. G., AND K. G. ADIYODI, 1972. Lipid metabolism in relation to reproduction and moulting in the crab, *Paratelphusa hydrodromous* (Herbst): Cholesterol and unsaturated fatty acids. *Indian J. Exp. Biol.*, in press.
- BARLOW, J., AND G. J. RIDGWAY, 1969. Changes in serum protein during the molt and reproductive cycles of the American lobster (*Homarus americanus*). *J. Fish. Res. Board, Can.*, 26: 2101-2109.
- CHIPPENDALE, G. M., AND B. A. KILBY, 1969. Relationship between the proteins of the haemolymph and fat body during development of *Pieris brassicae*. *J. Insect. Physiol.*, 15: 905-926.
- DRACH, P., 1939. Mue et cycle d'intermue chez les Crustacés Décapodes. *Ann. Inst. Oceanog. Paris*, 19: 103-391.
- GOMEZ, R., AND K. K. NAYAR, 1965. Certain endocrine influences in the reproduction of the crab, *Paratelphusa hydrodromous*. *Zool. Jahrb. Abt. Physiol.*, 71: 694-701.
- HUGGINS, A. K., AND K. A. MUNDAY, 1968. Crustacean metabolism. Pages 271-378 in O. Lowenstein, Ed., *Advances in Comparative Physiology and Biochemistry*, Vol. 3. Academic Press, New York.
- KERR, M. S., 1969. The hemolymph proteins of the blue crab, *Callinectes sapidus*. II. A lipoprotein serologically identical to oocyte lipovitellin. *Develop. Biol.*, 20: 1-17.
- KURUP, N. G., AND B. T. SCHEER, 1966. Control of protein synthesis in an anomuran crustacean. *Comp. Biochem. Physiol.*, 18: 971-973.
- LOCKWOOD, A. P. M., 1967. *Aspects of the Physiology of Crustacea*. W. H. Freeman and Company, San Francisco.
- MCWHINNIE, M. A., AND C. J. MOHRHERR, 1970. Influence of eyestalk factors, intermolt cycle and season upon ¹⁴C-leucine incorporation into protein in the crayfish (*Orconectes virilis*). *Comp. Biochem. Physiol.*, 34: 415-437.
- MILHAUD, G., 1964. Liver function tests. Pages 216-242 in Ch. Rouiller, Ed., *The Liver*, Volume 2. Academic Press, New York.
- MIYAWAKI, M., M. MATSUZAKI AND N. SASAKI, 1961. Histochemical studies on the hepatopancreas of the crayfish, *Procambarus clarkii*. *Kumamoto J. Sci.*, 5: 161-169.
- MIYAWAKI, M., AND S. TANOUE, 1962. Electronmicroscopy of the hepatopancreas in the crayfish, *Procambarus clarkii*. *Kumamoto J. Sci.*, 6: 1-12.
- NEILAND, K. A., AND B. T. SCHEER, 1954. The influence of fasting and of sinus gland removal on body composition on *Hemigrapsus mudus* (Part V of the hormonal regulation of metabolism in Crustacea). *Physiol. Comp. Oecol.*, 3: 321-326.
- OGURA, K., 1959. Midgut gland cells accumulating iron or copper in the crayfish, *Procambarus clarkii*. *Annot. Zool. Japon.*, 32: 133-142.
- OSER, B. L., 1965. *Hawk's Physiological Chemistry*. McGraw-Hill Book Company, New York.
- PEARSE, A. G. E., 1968. *Histochemistry, Theoretical and Applied, Vol. I*. London, J. and A. Churchill Ltd.
- SIKAKOTOS, A. N., 1960. The conjugated plasma proteins of the American cockroach. II. Changes during the molting and clotting processes. *J. Gen. Physiol.*, 43: 1015-1030.
- SKINNER, D. M., 1965. Amino acid incorporation into protein during the molt cycle of the land crab *Gecarcinus lateralis*. *J. Exp. Zool.*, 160: 225-234.
- VERNBURG, F. J., 1959. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. II. Oxygen consumption of whole organisms. *Biol. Bull.*, 117: 163-184.